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EVALUATION OF WOUND HEALING AND ANTIBACTERIAL PROPERTIES OF METHANOLIC ROOT EXTRACT OF *STEPHANIA DINKLAGEI* (ENGL.) DIELS

Sunday Ositadimma Udegbunam[1], Nnabuike Prince Igbokwe[1], Rita Ijeoma Udegbunam[1], Theophilus Okafor Nnaji[1], Madubuike Umunna Anyanwu[2*]

[1]Department of Veterinary Surgery, Faculty of Veterinary Medicine, University of Nigeria, Nsukka
[2]Microbiology Unit, Department of Veterinary Microbiology, Faculty of Veterinary Medicine, University of Nigeria, Nsukka.
*Email: madubuike.anyanwu@unn.edu.ng

Abstract

Background: *Stephania dinklagei* Diels (Engl.) is used in folkloric medicine in Southeastern Nigeria for the treatment of wounds and some bacterial-associated infections. This study evaluated the wound healing and antibacterial potential of *Stephania dinklagei* to validate or invalidate its folkloric use.
Materials and Methods: The minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of methanolic extract of *S. dinklagei* root (MESDR) against *Pseudomonas aeruginosa*, *Bacillus subtilis*, *Staphylococcus aureus*, *Escherichia coli* and *Klebsiella* spp. was determined by macro broth dilution. The extract at 20% and 10% were dosed orally to rats at 300mg/kg body weight (bw) in incision and dead space wound healing model to determine wound tensile strength and granulation tissue weight, respectively. The same extract concentrations were applied topically in excision wound model to determine the rate of wound contraction and epithelialization. Activities of superoxide dismutase (SOD) and catalase (CAT), and the levels of total protein (TP), malondialdehyde (MAL), hydroxyproline (HYP) and hexosamine (HEX) in excision wound biopsies were determined at days 7 and 14 post-wounding (pw). In the excision wound model, the extract concentrations were compared with gentamicin sulphate.
Results: The MIC of *S. dinklagei* extract against *P. aeruginosa*, *S. aureus*, *B. subtilis*, *E. coli* and *Klebsiella* spp. were 8mg/ml, 3 mg/ml, 5mg/ml, 6mg/ml and 7mg/ml, respectively, while the corresponding MBC were 10 mg/ml, 5 mg/ml, 7mg/ml, 8mg/ml, and 9 mg/ml, respectively. The 20% extract gave significantly ($P<0.05$) higher tensile strength and granulation tissue weight than the 10% and gentamicin sulphate. Wound contraction and epithelialization occurred significantly ($P<0.05$) better and faster in wounds of animals treated with the 20% extract and gentamicin sulphate compared to those treated with 10% extract. TP of animals treated with 20% extract and those treated with the reference drug did not vary significantly ($P>0.05$) at day 14 pw. SOD and CAT activities, and MDA and HEX level of all the groups did not vary significantly ($P>0.05$) at day 14 pw. HYP level of the extract-treated groups significantly ($P<0.05$) decreased against the control. No significant difference existed in HYP level between the extract-treated groups.
Conclusions: *S. dinklagei* possess antibacterial and wound healing properties which are comparable to those of gentamicin sulphate.

Key words: *Stephania dinklagei*, wound healing, antibacterial, antioxidant

Introduction

Wound is a break in the normal continuity of the skin which results in disruption in the cellular, anatomic and functional continuity of the body (Heydrari et al., 2011). Toxins resulting from surgical sepsis, infections and immune-suppressive agents have been incriminated in cutaneous wounds formation (Nguyen et al., 2009). Wound healing is a complex self-initiated process by which a disrupted organ/tissue repairs itself after injury (Nilani et al., 2011). Wound healing consists of four overlapping processes viz: granulation, fibroblast proliferation, collagenization and epithelialization. These processes occur in organized cascade culminating in restoration of anatomical continuity and function (Savunen and Viljanto, 1992; Shivhare et al., 2010). A major impediment to wound healing is contamination of wounds by bacteria (Darr et al., 1994). The emergence of wound-contaminating bacteria which are resistant to most conventional antibacterial agents and whose infections result in septicemia, toxemia, and sometimes death, have worsened the case (Akinyemi et al., 2005; Mboto et al., 2009; Abu-Al-Basal, 2010). Both Gram-positive (e.g. staphylococci, enterococci, *Pseudomons aeruginosa*, *Bacillus* spp., *Corynebacterium* etc) and Gram-negative (e.g. *Escherichia coli*, *Klebsiella* spp., *Proteus* spp., etc) bacteria constitute wound contaminants (Mboto et al., 2009; Abu-Al-Basal, 2010). The wound contaminating bacteria and infiltrating phagocytes release reactive oxygen species (ROS) (also called toxic oxidants or free radicals) which cause oxidative stress resulting in further damage to the wounded tissues thereby impeding healing process (Darr et al., 1994; Chen et al., 2012). Medical professionals use drugs that possess antibacterial and antioxidant activities in wound management (Udegbunam et al., 2014). But, the efficacies of orthodox drugs in wound management are no longer assured. Detrimental side effects in some individuals treated with orthodox wound healing drugs have been reported (Barnard et al., 2012). High cost of orthodox drugs is also a factor that has consistently lowered their patronage by the public (Udegbunam et al., 2013). The development and use of alternative and complementary substances to which resistant bacteria are still susceptible to, and which elicits little or no side effect has garnered public attention globally (Kunle et al., 2012).

In folkloric medicine, medicinal plants are used in the preparation of decoctions which are applied on skin wounds to enable healing to take place. The efficacies of these plants in wound healing and control of microbial growth have been experienced and passed on from one generation to the other (Akinyemi et al., 2005). *Stephania dinklagei* (Engl.) Diels (Family Merispermaceae) is one of the medicinal plants widely used in Southeast

Nigeria for treatment of wounds (Grace, 2008; Udegbunam et al., 2012). It is a slender climber growing in dense humid rain forest in West Africa (Grace, 2008; Iwu et al., 2014). It has rigid swollen stem and warty base. Its leaves are broadly oval to sub-orbicular and are of 15cm in diameter. It produces greenish flowers on old leafless branches (Iwu et al., 2014). Traditional practitioners in Southeastern Nigeria, including those in Nsukka, claim that the root part of *S. dinklagei* are used in form of poultices and decoction to treat several ailments such as sugar disease, mental illness, dysmenorrhoea, inflammatory conditions, headache, skin wounds, breast sores and leprosy (Grace, 2008; Semwal *et al.*, 2010; Udegbunam *et al.*, 2012; Nwaehujor et al., 2013; Iwu et al., 2014). In the treatment of wounds, the root decoction is drunk and the paste applied externally. Scientific investigations have validated some of the claims such as anti-diabetic (Nwaehujor et al., 2013), anti-inflammatory (Udegbunam et al., 2012), non-enzymatic antioxidant (Udegbunam et al., 2012; Nwaehujor et al., 2013) and sedative (Akubue et al., 1983; Semwal et al., 2010) effects of *S. dinklagei*. No study has evaluated the wound healing and antibacterial properties of *S. dinklagei*.

Moreover, preliminary phytochemical screening has also revealed the presence of alkaloids, tannins, terpenoids and flavonoids in *S. dinkglagei* root extracts of different solvents (Udegbunam et al., 2012). Specific chemical compounds in *S. dinklagei* as documented by Semwal et al., (2010) include liriodenine, dicentrinone, (+)-corydine, (+)- idocorydine, (−)-roemerine, N-methylliriodendronine, 2-O,N-dimethylliriodendronine, aloe-emodin, isocorydine, aporphines, atheroospermidine, tephalagine and dehydrostephalagine. Previous works by other researchers have shown that plant extracts that contain flavonoids, alkaloids and tannins promote wound healing by exhibiting antibacterial and antioxidant activities (Saeed et al., 2012; Ekren et al., 2013; Tsala et al., 2014). Since these phytoconstituents are reported to be contained in *S. dinklaglei* root extract, it may possess wound healing and antibacterial properties. The objective of this study therefore was to evaluate the antibacterial and wound healing properties of methanolic root extract of *S. dinklagei*.

Methods

The experimental protocols used in this study was approved by the Ethics Committee of the University of Nigeria, Nsukka and conforms with the guide to the care and use of animals in research and teaching of University of Nigeria, Nsukka, Enugu State Nigeria.

Animals

A total of 80 8-week-old male albino *Wistar* rats weighing between 130 and 156 g were obtained from the laboratory animal unit, Faculty of Veterinary Medicine, University of Nigeria, Nsukka. They were fed on commercial growers mash (Vital feeds®) and water was provided *ad libitum*. These rats were acclimatized for 2 weeks in the animal house at the Department of Veterinary Surgery, University of Nigeria, Nsukka.

Plant Collection and Identification

Fresh roots of *Stephania dinklagei* (Eng.) Diels were collected from Obollo Afor town in Nsukka Local Government Area Enugu State, Nigeria, in the month of May, 2014 and were identified at the International Center for Ethno-medicine and Drug Development (InterCEDD), Nsukka, by a plant taxonomist, Mr. A. Ozioko. Samples were registered with a voucher specimen number SDMN/16/14 and deposited in the center's herbarium.

Extraction

A kilogram of the *S. dinklagei* roots were cut into smaller pieces, air dried at room temperature for 2 weeks, and then pulverized using the laboratory grinding machine at the Department of Crop Science, University of Nigeria, Nsukka. The pulverized roots were macerated in 80% methanol for 48 hours with intermittent vigorous shaking at every 2 hours. After 48 hours, the mixture was filtered and the extract concentrated using a rotary evaporator set at 40°C. The dried extract was weighed and the percentage yield calculated. The extract was then stored at 4°C in a refrigerator before use.

Pathogens and Preparation of Inocula

The bacterial organisms - *Pseudomonas aeruginosa*, *Stapylococcus aureus*, *Bacillus subtilis*, *Escherichia coli* and *Klebsilla* spp used in this study were collected from the Department of Pharmaceutics, University of Nigeria, Nsukka. They were clinical wound isolates from patients in Nsukka, Nigeria, fully identified and maintained on nutrient agar slope at 4°C at the Department of Pharmaceutical Microbiology Laboratory, University of Nigeria, Nsukka. Prior to use, the bacterial organisms were sub-cultured on sterile nutrient agar plate, incubated aerobically at 37°C for 24 hours. Colonies of each organism were homogenized in sterile phosphate buffered saline (PBS) and the turbidity adjusted to correspond to 0.5 McFarland's turbidity standard (equivalent to 1×10^8 cfu/ml). The standardized broth cultures were kept at 4°C until needed.

Acute Toxicity Test

Thirty adult rats were randomly divided into six groups of five animals per group. The animals were deprived water for 16 hours before administration of the extract. The increasing doses of the extract 200, 400, 600, 1000 and 2000 mg/kg body weight dissolved in sterile normal saline was administered orally to the test groups, respectively, using a ball-tipped intubation needle fitted onto a syringe. The last group received 1ml/kg of sterile normal saline and served as the control. The rats were allowed access to food and water *ad libitum* and were observed for 48 hours for behavioural changes and death. The time of onset, intensity, and duration of these symptoms, if any, was recorded.

Phytochemical Analysis for Bioactive Substances

The extract was screened for the presence of bioactive components: glycosides, tannins, saponins, alkaloids and flavonoids following the methods of Trease and Evans (1983).

Determination of Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC)

MIC of the extract was determined by macro broth dilution following the method described by Boron and Finegold (1990). Dilution of *S. dinklagei* extract was made to various concentrations of 1, 2, 3, 4, 5, 6, 7, 8, 9 and 10 mg/ml in series of test tubes. Equal volume of the extract and sterile brain heart infusion broth (Oxoid®, Bangistoke UK) were mixed. Specifically, 0.1 ml of standardized inoculum (1×10^8 cfu/ml) of each of the test organism was added to each tube. The tubes were incubated aerobically at 37°C for 24 hours. Two control tubes were maintained for each test batch. These included antibiotic control (tube containing extract and the growth medium without inoculum) and organism control (the tube containing the growth medium, normal saline and the inoculum). The lowest concentration (highest dilution) of the extract that produced no visible bacterial growth (no turbidity) when compared with the control tubes were regarded as MIC. However, the MBC was determined by sub-culturing the test dilution on to a fresh drug-free nutrient agar (Oxoid®, Bangistoke UK) and incubated further for 24 hours. The highest dilution that yielded no single bacterial colony on a solid medium was taken as MBC.

Wound Healing Studies
Incision Wound Model

Eighteen rats were anesthetized by injecting intramuscularly with 10 and 50 mg/kg bw of xylazine hydrochloride and ketamine hydrochloride, respectively. Incision wound was created following the procedure described by Rathi et al. (2004). Briefly, under general anesthesia, dorsum of the animals were shaved thoroughly and prepared for aseptic surgery. Paravertebral skin incisions (5 cm in length) were made on the animals using sterile scalpel blade. The incisions were sutured using size 2/0 silk thread. Then, the rats were randomly assigned into three treatment groups consisting of 6 animals per group and treated as follows: Groups A and B were dosed orally with 300 mg/kg bw of 20% and 10% of the MESDR once daily, respectively, while group C was similarly given 1 ml/kg body weight (bw) of sterile normal saline. The animals were treated daily for a period of 7 days. Sutures were removed at day 8 post-wounding (pw). The wound tissue breaking strength was determined at day 10 pw using the constant water flow technique described by Morton and Malone (1992).

Dead Space Model

Eighteen rats were randomly assigned into 3 groups of 9 animals per group. They were anaesthetized by injecting intramuscularly with 10 and 50 mg/kg bw of xylazine hydrochloride and ketamine hydrochloride, respectively. Dead space wound was created following the procedure described by Rathi et al. (2004). Briefly, under general anesthesia, subcutaneous dead space wounds were created in the region of the axilla by making a pouch through a small nip in the skin. Granulation tissue formation was induced by implanting one 30 mg sterile cotton pellets in each axilla. The wounds were sutured using size 2/0 silk and mopped with alcoholic swab. The animals were grouped and then placed individually in a clean and disinfected metal cage to avoid them licking or biting each other's wound. groups D and E were administered orally with 300 mg/kg bw of 20% and 10% of the MESDR once daily, respectively, while group F was similarly given 1 ml/kg of sterile normal saline for 8 days. At day 10 pw, rats were euthanized, the cotton pellets together with the granulation tissues carefully dissected out, dried in a hot air oven at 40°C for 24 hours and weighed. Weight of the granulation tissue was obtained by subtracting the post-drying weight from the pre-implantation weight.

Excision Wound Model

Twenty four rats were anaesthetized by injecting intramuscularly 10 and 50 mg/kg bw of xylazine hydrochloride and ketamine hydrochloride, respectively. Under general anaesthesia, dorsum of the rats were shaved and disinfected. Then, full thickness 30 mm circular wound was made on the dorsal thoracic region following the method described by Dash et al. (2001). Post wounding, the rats were randomly assigned into 3 groups of 8 animals per group and treated as follows: groups I and II were treated topically with 300mg/kg of 20% and 10% MESDR, respectively, while groups III was treated with 1% gentamicin sulphate ointment and served as the control. The animals were housed individually in clean and dust-free cages in the Department of Veterinary Surgery, University of Nigeria, Nsukka to avoid wound biting and/or licking and treated daily with their respective extract concentrations or drug until complete healing occurred.

Determination of Percentage Wound Contraction of Excision Wound

Percentage wound contraction of the excision wound was determined following the procedure described by Chah et al. (2006) and Ezike et al. (2010). Briefly, at days 4, 8, 12, 16 and 20 post infliction of excision wound, wound diameter were manually traced on a transparent white tracing paper by outlining the wound edge with a fine-tip permanent marker. After the tracing of wound diameter of each animal, the tracing paper was appropriately labeled with the group number, rat identity and date. The area within the lines of each tracing was determined by placing the tracing paper on a $1mm^2$ graph sheet and traced out. The squares were counted and the area recorded. The degree of wound contraction was determined by subtracting the total wound area of each tracing day from the area of the initial tracing. Percentage wound contraction was then calculated as described by Chah et al. (2006).

Wound Epithelialization

Period of wound epithelialization (i.e. the number of days required for the scar to fall off leaving no raw wound and epithelium covered the entire wound) was determined following the method described by Okoli et al. (2009).

Assay of Biochemical Parameters in Excision Wound Biopsy

Wound biopsy specimens were collected from a rat in each group at days 7 and 14 pw. Biopsy specimens were preserved in 10% phosphate buffered saline and used for biochemical assay of superoxide dismutase (SOD) following the method described by Misra and Fridovich (1972), catalase (CAT) using the method described by Takahara et al. (1960), malondialdehyde (MAL) level using method described by Bueg and Aust (1978), hydroxyproline (HYP) following the procedure described by Reddy and Enwemeka (1996), hexosamine (HEX) as per Elson and Morgan (1933) and total protein (TP) determined following the protocol described by Tietz (1995).

Statistical Analysis

Data obtained were summarized as mean ± standard error of mean. Mean values of wound breaking strength, granulation tissue weight, percentage wound contraction, wound epithelialization time and the values of the biochemical parameters for different groups were compared using one-way Analysis of Variance. Duncan multiple range test was used to separate variant means. $P<0.05$ was considered significant.

Results

Extraction

The MESDR had aromatic smell and was brownish-green in colour. The percentage yield was 7.74% w/w material.

Acute Toxicity Test

Administration of MESDR extract in normal saline to rats even at the highest dose of 2000 mg/kg bw did not produce any death in the treated groups. No sign of acute toxicity was also observed.

Phytochemical Analysis

Preliminary phytochemical analysis of MESDR qualitatively revealed the presence of alkaloids, tannins, saponins and flavonoids (Table 1).

Table 1: Phytochemical analysis of methanolic extract of *Stephania dinklagei* root

Phytoconstituent	Amount
Steroids	-
Glycosides	-
Saponins	++
Alkaloids	+++
Sugar	-
Phenol	++
Tannins	+++

Keys: +++ = appreciable amount, ++ = moderate amount, − = completely absent

Minimum Inhibitory and Bactericidal Concentration of MESDR on Wound Isolates

The MIC values obtained for the MESDR against *P. aeruginosa*, *S. aureus*, *B. subtilis*, *E. coli* and *Klebsiella* spp. was 8mg/ml, 3 mg/ml, 5 mg/ml, 6mg/ml and 7mg/ml, respectively, while the corresponding MBC were 10 mg/ml, 5 mg/ml, 7mg/ml, 8mg/ml, and 9 mg/ml, respectively (Table 2)

Table 2: Minimum inhibitory and bactericidal concentrations of methanolic extract of *Stephania dinklagei* root on tested bacterial organisms

Bacterial organism	MIC (mg/ml)	MBC (mg/ml)
Pseudomonas aeruginosa	8	10
Staphylococcus aureus	3	5
Bacillus subtilis	5	7
Escherichia coli	6	8
Klebsiella spp.	7	9

Keys: MIC: minimum inhibitory concentration; MBC: minimum bactericidal concentration

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Incision Wound

The result of the effect of MESDR on breaking strength of the healed wound showed significantly (P< 0.05) higher wound breaking strength in animals in MESDR treated groups when compared with the control (Table 3).

Table 3: Wound tissue breaking strength in rats post infliction of incision wound

Group (treatment)	Mean±sem wound tissue breaking strength (g)
A (300 mg/kg bw of 20% MESDR)	598.86±83.2[a]
B (300 mg/kg bw of 10% MESDR)	395.86±59.3[b]
C (Control)	224.46±40.9[c]

Keys: bw: body weight, MESDR: methanolic extract of *Stephania dinklagei* root; sem: standard error of mean
Different superscripts across a column indicate significant difference in means at P<0.05

Dead Space Wound

The result of the effect of MESDR on granulation tissue weight showed that dry granulation tissue weight of animals in group D (20% MESDR) was significantly (P<0.05) higher when compared against that of animals in group E (10% MESDR) and the control (Table 4). There was no significant difference (P>0.05) in granulation tissue weight of animals in groups E and the control.

Table 4: Granulation tissue weight in rats post infliction of dead space wound

Group (treatment)	Mean±sem of granulation tissue weight (g)
D (300 mg/kg bw of 20% MESDR)	87.7±9.51[a]
E (300 mg/kg bw of 10% MESDR)	73.2±9.36[ab]
F (Control)	63.8±6.42[b]

Keys: bw: body weight, MESDR: methanolic extract of *Stephania dinklagei* root; sem: standard error of mean
Different superscripts across a column indicate significant difference in means at P<0.05

Excision Wound:
Percentage Wound Contraction

The percentage wound contraction of all the groups did not vary significantly (P>0.05) at 4 dpw (Table 5). At days 8 and 12 pw, percentage wound contraction of MESDR treated groups (I and II) were significantly lower when compared to the control. At days 16 and 20, percentage contraction of group I did not vary significantly (P>0.05) with that of the control, but was significantly higher than group II.

Table 5: Percentage rate of wound contraction in rats post infliction of excision wound

Group (treatment)	Mean±sem wound contraction (%) at days post wounding				
	4	8	12	16	20
I (300mg/kg 20% MESDR)	10.81±1.62[a]	37.49±3.69[b]	71.78±2.69[b]	94.5±2.16[ab]	100±0.00[a]
II (300mg/kg 10% MESDR)	11.2±2.19[a]	41.19±3.34[b]	70.83±2.19[b]	91.45±1.81[b]	96.83±1.26[b]
III (Gentamicin sulphate)	13.57±1.45[a]	77.5±1.67[a]	77.5±1.67[a]	98.98±1.02[a]	100±0.00[a]

Keys: bw: body weight, MESDR: methanolic extract of *Stephania dinklagei* root; sem: standard error of mean
Different superscripts across a column indicate significant difference in means at P<0.05

Epithelialization occurred between day 8 and 14. Epithelialization occurred significantly (P<0.05) faster in animals in group I than those in group II (Table 6). Animals in both MESDR treated groups (I and II) had significantly (P<0.05) longer epithelialization time compared with those in the control group.

Table 6: Wound epithelialization time of rats post infliction of excision wound

Group (treatment)	Mean±sem (days) epithelialization time post wounding
I (300mg/kg 20% MESDR)	10.66±0.92[a]
II (300mg/kg 10% MESDR)	13.66±1.48[b]
III (Gentamicin sulphate)	8.33±0.91[c]

Keys: bw: body weight, MESDR: methanolic extract of *Stephania dinklagei* root; sem: standard error of mean
Different superscripts across a column indicate significant difference in means at P<0.05

Effect of MESDR on Biochemical Parameters in Excision Wound Biopsy

Results of the effect of MESDR on assessed biochemical parameters in excision wound biopsy at days 7 and 14 post wounding are presented in tables 7 and 8, respectively. At day 7 pw, the total protein (TP) in wound biopsy of animals in the MESDR treated groups (I and II) significantly (P<0.05) decreased compared to the control (Table 7). At day 14, TP of group I did not vary significantly (P>0.05) with the control (Table 8). At day 7 pw, no significant difference (P>0.05) occurred in malondialdehyde (MDA) level in wound biopsy of animals in group I and the control, but MDA of both groups decreased significantly (P<0.05) when compared with group II (Table 7). At day 14 pw, MDA level of all the groups did not vary significantly (P>0.05) (Table 8). No significant difference (P>0.05) existed in the superoxide dismutase (SOD) and catalase

activities, and hexosamine level among all the groups throughout the study period (Tables 7 and 8). The hydroxyproline (HYP) level of group II was significantly (P<0.05) higher when compared with group I and the control at day 7 pw (Table 7). At day 14 pw, the HYP level of MESDR treated groups (I and II) significantly (P<0.05) decreased on comparison with the control (Table 8). No significant difference existed in HYP level between both MESDR treated groups.

Discussion

In this study, the wound healing property of methanolic extract of *Stephania dinklagei* root (MESDR) was determined using the incision, dead space and excision wound models, which are the standard models for evaluating wound healing potentials of plant extracts (Al-Henhena et al., 2011; Tsala et al., 2014); while the antibacterial potential of the plant extract was determined using a standard *in vitro* quantitative method of macro broth dilution (Jorgensen and Ferraro, 2009). The fact that the MIC values obtained for the tested bacterial organisms (*P. aeruginosa* 8mg/ml, *S. aureus* 3 mg/ml, *B. subtilis* 3 mg/ml, *E. coli* 6mg/ml and *Klebsiella* spp. 7mg/ml) were within the tested concentration suggest that *S. dinklagei* inhibited the growth of the organisms at the respective extract concentrations. This is further supported by the corresponding MBC values (*P. aeruginosa* 10 mg/ml, *S. aureus* 5 mg/ml, *B. subtilis* 7mg/ml, *E. coli* 8mg/ml, and *Klebsiella* spp 9 mg/ml). The antibacterial activity of the plant extract may be attributed to the phytoconstituents especially flavonoids and alkaloids present in the extract as revealed by the phytochemical analysis.

Table 7: Effect of methanolic extract of *Stephania dinklagei* root on biochemical parameters in wound biopsy at day 7 post infliction of excision wound

Parameter	Mean±sem level in wound biopsy at day 7 post wounding for each group (treatment)		
	I (300mg/kg 20% MESDR)	II (300mg/kg 10% MESDR)	III (Gentamicin sulphate)
Total protein (mg/dl)	1.28±0.16^{b}	0.85±0.1^{b}	2.98±0.36^{a}
Malondialdehyde (mg/dl)	0.58±0.07	0.76±0.04	0.6±0.07
Superoxide dismutase (IU)	87.18±10.61	87.18±10.61	94.91±11.57
Catalase (IU)	0.58±0.07	0.52±0.06	0.61±0.75
Hydroxyproline (mg/g tissue)	0.37±0.04^{b}	19.00±0.12^{c}	0.68±0.82^{a}
Hexosamine (mg/g tissue)	0.31±0.04	0.27±0.33	0.37±0.46

Keys: MESDR: methanolic extract of *Stephania dinklagei* root; sem: standard error of mean
Different superscripts across a row indicate significant difference in means at P<0.05

Table 8: Effect of methanolic extract of *Stephania dinklagei* root on biochemical parameters in wound biopsy at day 14 post infliction of excision wound

Parameter	Mean±sem level in wound biopsy at day 14 post wounding for different group (treatment)		
	I (300mg/kg 20% MESDR)	II (300mg/kg 10% MESDR)	III (Gentamicin sulphate)
Total protein (mg/dl)	1.93±0.23^{ab}	1.28±0.16^{b}	3.98±0.48^{a}
Malondialdehyde (mg/dl)	0.32±0.08	0.31±0.07	0.35±0.08
Superoxide dismutase (IU)	92.31±11.24	89.74±10.92	94.91±11.57
Catalase (IU)	1.91±0.23	1.89±0.23	2.29±0.28
Hydroxyproline (mg/g tissue)	0.68±0.08^{a}	0.29±0.23^{b}	0.69±0.08^{a}
Hexosamine (mg/g tissue)	0.78±0.09	0.67±0.08	0.89±0.11

Keys: MESDR: methanolic extract of *Stephania dinklagei* root; SEM: standard error of mean
Different superscripts across a row indicate significant difference in means at $p < 0.05$

Reports showed that plant extract which contained flavonoids and alkaloids exhibited antibacterial activities (Goncalves et al., 2009; Ekrem et al., 2013). The antibacterial result of this study suggests that *S. dinklagei* extract inhibited the growth of *S. aureus* and *B. subtilis* better than the other tested organisms. Variation in MIC and MBC of the extract against the organisms may be due to differences in their resistance to the antibacterial phytoconstituents in the plant extract. The highest MIC and MBC for *P. aeruginosa* is not suprising, because, apart from being a septic wound isolate, most pseudomonal isolates are inherently resistant to most antibacterial agents including plant extracts that possessed antibacterial activity (Li et al., 1994; Udegbunam et al., 2015).

Release of reactive oxygen species (ROS) by the infiltrating phagocytes in wound bed as well as contaminating bacteria and those released by the skin following ultraviolet exposure results in oxidative stress (Trenam et al., 1992). This further damage to wounded skin tissue caused by lipid peroxidation is evidenced by increased malondialdehyde level in the wound tissue (Trenam et al., 1992; Darr et al., 1994). In this study, the activities of enzymatic antioxidants superoxide dismutase (SOD) and catalase (CAT), and levels of malondialdehyde (MAL) in excision wound biopsies were assayed to determine the superoxide (O_2^-) and hydrogen peroxide (H_2O_2) radical scavenging (antioxidant) activity of *S. dinklagei* extract, and degree of lipid peroxidase activity (cellular lipid peroxidation), respectively. Superoxide radical (SO_2^-) although a weak oxidant, is a major biological source of ROS such as dangerous hydroxyl radical and singlet oxygen both of which contribute to oxidative stress and tissue damage (Alves et al., 2010; Khan et al., 2012). SO_2^- is scavenged and degraded by SOD (an antioxidant) to hydrogen peroxide (H_2O_2) which is then degraded by CAT to water and oxygen molecules which are non-toxic to tissues (Singleton, 2005; Khan et al., 2012). Non-significant difference in the activities of SOD and CAT among the groups throughout the study period, suggest that both concentrations of the extract enhanced wound healing effect by eliciting antioxidant effect (i.e. SO_2^- and H_2O_2 scavenging) comparable to that of gentamicin sulphate. This may account for the

non-significant difference in MDA level among the groups. The antioxidant effect of the extract observed in this study could be attributed to its phytochemical constituent especially the tannins. Plants extract that contained alkaloids, flavonoids and tannins have been widely reported to promote wound healing by eliciting antioxidant effect (Gonclaves et al., 2009; Saeed et al., 2012; Ekren et al., 2013). Antioxidants have been reported to play a significant role in wound healing process (Martin, 1996). Therefore, the observed antioxidant effect may partly be a mechanism by which the MESDR promoted wound healing. The finding of enzymatic antioxidant effect by methanolic *S. dinklagei* root extract in this study, corroborates with Nwaehujor et al. (2013) report which stated that the extract possessed non-enzymatic (i.e. diphenylpicrylhydrazyl [DPPH] scavenging activity) property.

Wound contraction which is the process of shrinkage of wound area, depends on the reparative abilities of the tissue, type and extent of the tissue damage (Priya et al., 2004). Therefore, the significant decrease in percentage wound contraction when the MESDR-treated groups were compared with the control from day 12-16 post-wounding (pw), tend to suggest that the reference drug (gentamicin sulphate) promoted wound healing better than both concentrations (20 and 10%) of the extract used. But, the lack of significant difference in percentage wound contraction between group I (treated with 20% MESDR) and the control by day 20 pw (both groups had 100% contraction) suggests that the 20% MESDR promoted wound healing at a rate comparable to that of gentamicin sulphate. The significantly shortest epithelialization time of the control group when compared with the MESDR-treated groups, suggest that the reference drug may have enhanced collagen deposition more than both concentrations of the extract. The significant difference observed in the increase in percentage wound contraction and epithelialization in both groups of rats treated with 20% and 10% MESDR respectively suggests that the 20% concentration elicited better wound healing effect than the 10%. This suggests that the extract exhibited a concentration-dependent activity. It also suggests that the more the availability of the phytochemicals the better the effect (Udegbunam et al., 2015). Therefore, it is possible that the 20% MESDR promoted wound contraction more than the 10% concentration but not the reference drug. Plants with antibacterial and antioxidant properties enhance wound healing by accelerating wound contraction and re-epithelialization (Calabresse et al., 2000; Ezike et al., 2010; Okoli et al., 2007).

The amount of fibroblast proliferation, collagen synthesis and neo-vascularization determines the tensile/wound breaking strength (Habibipour et al., 2003). The principal strength of a healed tissue is embodied on its collagen content which supports the extracellular tissue (Kumar et al., 2006). The fact that the wound breaking strength of group I (20% MESDR) significantly increased when compared with group II and the control, suggest that 20% extract concentrations may have promoted collagen synthesis, fibroblast proliferation and neo-vascularization better than the 10% concentration and the reference drug. But the best method of assessing collagen turnover or synthesis is measuring the hydroxproline (the major amino acid component of collagen) and hexosamine level in wound biopsy (Chen et al., 2012). Increase in hydroxyproline content indicates increased collagen synthesis and invariably wound healing while increased hexosamine content reflects the stabilization of collagen molecules via enhanced electrostatic and ionic interactions (Ricard-Blum and Ruggiero, 2005; Chen et al., 2012). Thus, the significant difference in decrease in the HYP level of the MESDR-treated groups compared with the control suggests that the reference drug could have promoted collagen synthesis more than the extract concentrations used in this study. Non-significant difference in the hexosamine level of wound biopsy from animals in all the groups further suggest that the extract and the reference drug exerted comparable collagen stabilization and thus wound healing effect. Enhanced hydroxyproline and hexosamine synthesis provides strength to repaired tissue and stimulates healing (Chen et al., 2012).

Granulation tissue formed in a dead space wound contains modified macrophages, histological giant cells and undifferentiated connective tissue of mainly collagen (Bairy and Rao, 2001). Increase in granulation tissue in dead space wound is associated with enhanced collagen maturation and increased protein content as well as angiogenesis in the wound (Abu-Al-Basal, 2010). The fact that granulation tissue weight of animals in group D (treated with 300mg/kg bw of 20% MESDR) was significantly higher when compared against that of animals in group E (treated with 300mg/kg bw of 10% MESDR) and the control (treated with sterile normal saline) may also suggest that the 20% extract promoted more rapid collagen maturation than the 10% extract and the reference drug. Non-significant difference in granulation tissue weight of animals in groups E and the control suggests that even the 10% extract promoted maturation of collagen tissues at a rate comparable to that of the reference drug.

Total protein (TP) content is an indicator for the protein level and cellular proliferation of the wounded tissue (Teoh et al., 2012). Lack of statistical significance in TP level of group I (300mg/kg 20% MESDR) and the control at day 14 pw, further suggest that the extract promoted wound healing at a rate comparable to the reference drug. This may account for similar epithelialization rate observed between the groups. Significant difference in TP among the MESDR-treated groups at day 14 pw further suggest that the extract exhibited wound healing effect in a concentration-dependent manner.

Conclusion

This study has shown that methanolic extract of *S. dinklagei* root possesses antibacterial and wound healing properties which are comparable to those of gentamicin sulphate. The use of *S. dinklagei* root for wound treatment in folkloric medicine is thus validated. *S. dinklagei* root could be a potential source for a drug that would be useful in wound management. However, further studies that would involve cell biology and immunology is recommended.

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